A PRELIMINARY STUDY OF THE SPERMATOGENESIS OF BELOSTOMA (ZAITHA) FLUMINEA

By A. M. Chickering

The study of *Belostoma fluminea* was begun during the summer of 1914 at the University of Wisconsin under the direction of Prof. M. F. Guyer to whose help and criticism the writer owes much. I also wish to take this opportunity to express my gratitude to Prof. H. D. Densmore of Beloit College for the loan of expensive and indispensable apparatus.

MATERIAL AND METHODS

Belostoma fluminea is the common, giant water bug of our shallow ponds and slowly moving streams. This form is very common in the region around Madison, Wisconsin and has been taken in large numbers, especially during the month of July.

Nymphs and adult males, which are often taken bearing the egg-cluster on their backs, were used to obtain the necessary material. Some of the adults show the whole general history of the germ cells from spermatogonia to mature spermatozoa with great clearness. By careful study the seriation is placed beyond reasonable doubt by virtue of the arrangement of the cells in cysts in a nearly progressive series from one end of the testes to the other.

On account of their extreme delicacy and transparency some trouble was experienced at the beginning in removing the testes from their position in the abdominal cavity to the fixing fluid. Later, however, this was overcome by squirting the fixing fluid directly on to the testes after removal of the dorsal abdominal wall. After a few moments the organs stood out sharply and could be transferred easily to the final fixing fluid without injury.

The best results in fixing were obtained with Gilson's and Bouin's fluids.

The iron-hæmatoxylin method of staining has proven very satisfactory for this work. Other stains have been tried with

varying degrees of success but all the slides used in preparing this paper were stained with Haidenhain's iron-hæmatoxlyin. Sections were cut from four to six μ in thickness.

Smears have been almost uniformly unsuccessful. In a few cases smear slides of fair quality were obtained but as yet no careful study has been made of them. The method of preparation as described by Morse ('09) gave better results than any other. The testes are simply removed to a slide and the cysts pricked or teased gently. This allows the contents to run evenly over the slide which is afterwards dried and stained without the use of any fixing fluid.

The works cited at the end of this paper are only those which have been especially helpful to the writer in preparing this brief paper. No attempt has been made to append a complete list of papers on this subject.

SPERMATOGENESIS

I. Spermatogonial stages

The spermatogonial divisions are very numerous in this material, particularly so in the testes taken from nymphs. But usually the chromosomes are so crowded together in these division stages as to prevent any accurate counting or study of the separate chromosomes. In fact the writer has been able to find only four metaphase plates in which the chromosomes could be effectively studied. These were all found in the testes of a single specimen and are probably undergoing final division before synapsis.

The two cells shown in figures 1 and 2, have the chromosomes arranged in a very flat equatorial plate in a plane almost parallel to the plane of the section. Both show twenty-four chromosomes, and in each case there are four large bean-shaped chromosomes, eighteen of intermediate size and spherical or ellipsoid in shape, and two very small ones. Nearly all are connected to one or more of the others by means of delicate filaments.

The other two cells mentioned above show, respectively, twenty-three and twenty-two chromosomes. In the first case one of the small pair is missing and in the second both are missing. These are so small and the chromosomes so numerous that they

may be covered up easily and so escape detection. From the number and behavior of the chromosomes in the maturation divisions one would expect the spermatogonial or unreduced number to be twenty-four. The writer considers it fair to conclude from the evidence at hand that such is the case.

It is quite evident from inspection of figures 1 and 2, that three pairs of chromosomes may be identified at this stage. The xy-pair, which becomes so apparent in the second maturation division, is not distinguishable nor so far has it been possible to recognize more pairs of chromosomes in this stage.

II. Synaptic and Post-Synaptic Stages

It has thus far been found impossible to obtain satisfactory results with *Belostoma* in attempts to work out the history of the chromosomes during synapsis and the early prophase stages of the primary spermatocytes; hence no attempt will be made to describe these in any detail, only a brief outline being given. I hope, however, to obtain suitable smear material at a later date and make a careful study of the stages necessarily slighted now.

Immediately following the last spermatogonial division the chromosomes loosen up and flow together into a rather lightly staining, confused network (Fig. 3). This stage is of short duration, passing quickly into what is probably a spireme stage but since no suitable material is available no drawings have been made from this point to synizesis (Fig. 4). The threads making up the synaptic knot are so crowded and tangled that usually only the ends of the threads can be seen projecting beyond the heavily stained mass. The knot is usually placed at one pole of the nucleus and often a plasmosome is visible in these stages.

After the synizesis stage the threads quickly spread apart throughout the nucleus. They now appear very thick and heavy, and stain more deeply than in the preceding stages (Fig. 5-6). The plasmosome attains its maximum size during this stage and gradually diminishes and finally disappears during the early prophases.

In the later part of this period some nuclei appear to show a divided condition of the separate threads even in sections and in the best smears the threads are distinctly divided by a longitudinal cleft (Fig. 7). Usually in the sections there are shown

two threads which are much longer and broader than any of the rest, presumably corresponding to the two large chromosomes of the first division and made up of the four bean-shaped spermatogonial chromosomes.

No evidence has yet been adduced to show that the sex-chromosomes in *Belostoma* are present in the form of chromatic nucleoli during the growth period as described by Wilson ('12) in the spermatogenesis of *Lygæus* and *Oncopeltus* although the general history of the chromosomes seems to be very similar to that of the two forms mentioned.

Before the prophase stages begin there is interpolated a "confused" stage which results from a fading out and ultimate disappearance of the heavy, divided threads. This process gives rise to a nucleus which is traversed throughout by a loose network of chromatin material consisting of much finer, irregular threads the boundaries of which can not be made out. This goes on from Figure 8 until the network is reduced to an exceedingly confused condition. In this condition the threads stain so lightly and are so interlaced as to make it impossible to see the separate elements at all. This "confused" stage is of relatively long duration, but finally condensation starts and the network is reorganized into the tetrad rods, rings and V's (Fig. 9-9A).

III. Chromatoid Bodies

In the confused stage are *clearly* seen for the first time (further work seems likely to disclose their presence earlier) bodies which have been called the chromatoid bodies (Wilson '13 and Fasten '14).

The larger of these bodies which becomes very prominent in the division of the primary spermatocyte attracted my attention when the work on *Belostoma* was first started. It then seemed to be an x-chromosome and for a time the work was carried on with that idea. The reader will readily see the resemblance of figures 14-23 to several already published by numerous workers demonstrating the presence of an odd chromosome. In the case here presented this idea must be abandoned. The chromatoid bodies are seen in Fig. 8 outside of the nuclear membrane and therefore

not directly connected with the chromatin elements. They are never found within the spindle although they are sometimes so close to the group of chromosomes that they are indistinguishable in polar views of metaphase plates, and in this way sometimes are very confusing in taking chromosome counts. They do not occur in all cells and when present behave irregularly. With iron-hematoxylin they are stained like the chromosomes.

IV. Primary Spermatocytes

In the early tetrad stages of the chromosomes two rings are very large and prominent. They are in practically every cell and seem to give rise to the two large chromosomes of the mature spermatocyte by a decrease in the diameter and a closing up process. There are other smaller rings sometimes showing but they do not seem to be constant. The rods seem to shorten and thicken to form the final chromosomes; the same process probably takes place with the Vs, and the rings apparently close up to form such tetrads as shown in Figs. 10-11. Such figures are very common in my material and may be favorable for working out the details of the changes taking place here.

All of these figures finally condense into dumb-bell shaped bodies (Fig. 12). This shape is retained throughout the first division, the constriction marking the plane of cleavage.

Very shortly after the stage shown in the last figure the nuclear membrane begins to break down, the spindle forms at the two poles, the chromosomes take up a position in the equatorial plane and the cell is ready for division.

The chromatoid bodies remain of about the same size until the spindle begins to be formed and then a decided increase in the size of at least one of them is plainly seen (Fig. 13-15.)

The stages from this point onward show with diagrammatic clearness. There are thirteen chromosomes in the equatorial plate of the primary spermatocyte. This number is one more than half the spermatogonial number. The arrangement of chromosomes is inconstant, no two plates showing the same placing with respect to each other. There are two large, ten intermediate and one very

small chromosomes, they all divide equally in this division, carrying over thirteen in every case to the secondary spermatocytes (Figs. 14-24).

These stages have been carefully searched to find the sex-chromosomes and to determine their behavior but without success.

The chromatoid bodies are seen here (Figs. 14-24) at their maximum size. Usually there is only one present, but quite often there are two and more rarely three. In a few cases four have been seen. Occasionally one or more of the number has an irregular shape (Figs. 14, 20 and 22).

When division takes place these bodies are not apportioned evenly but may all be retained in a single secondary spermatocyte, or one or more may go into each. No regularity seems to obtain in the distribution of these bodies. Given three or four of different sizes and a large number of types of secondary spermatocytes may be derived if classified on the basis of the kind and number of chromatoid bodies they possess.

V. The Interkinesis

It seems to be a well established fact that in the interkinesis there is no more than a slight pause between the telophase of the first and the prophase of the second divisions. The chromosomes are much crowded together, but enough is evident to show that they retain their individuality and are not in any degree reorganized into a nucleus (Fig. 25). The centrosomes also divide in late anaphase and are already well moved apart, accompanied by small spindles, in the late telophase.

Whether the grouping of chromosomes is changed in this stage is uncertain, but the probability is that the new grouping assumed in the next metaphase takes place during the prophases of the same division.

VI. Secondary spermatocytes

In the prophases of this division the spindle is very rapidly built up while the chromosomes which were so crowded before now spread apart and assume *a grouping very different from that in the first division.* Eleven of the chromosomes are arranged in a circle at the equator of the spindle while two remain in the center

of the circle forming an xy-pair of sex-chromosomes. In polar views only twelve chromosomes are usually visible (Figs. 26-28). Lateral views of the same stage show however, that what appears to be a single chromosome in polar views is in reality the xy-pair, the members of which are united end to end. (Fig. 29).

All of the stages through inter-kinesis leading up to the metaphase in which the sex-chromosomes are seen united have been studied to see how complete the conjugation between the two is effected, and such a process seems to be limited to a simple end to end union as described above. This union exists only for a short time before the final separation and seems to take place while the chromosomes are rearranging themselves in their new grouping during the later prophase stages.

By a comparative study of Figs. 23-25, and 29-30, it will be seen readily that a rotation of each chromosome through about ninety degrees takes place while the spindle is being formed, so that the long axis of each becomes parallel instead of perpendicular to the long axis of the spindle. A rotation of the entire group may also take place as described for *Oncopeltus* and *Lygæus* (Wilson '10) although it seems more probable that the virtual rotation is accomplished by the relative change in position of the separate chromosomes as they assume their new grouping.

In the latter part of this period the sex-chromosomes have separated and are on their way to the opposite poles of the spindle before the others have even started to divide at all (Fig. 30). This aptitude for the sex-chromosomes seems to be a common phenomenon and has been described by numerous workers.

The chromatoid bodies present about the same appearance as in the primary spermatocytes. There are many cells without any chromatoid body, many more with only one large one, and others with two or three.

VII. The Spermatids

When division of the secondary spermatocytes takes place twelve chromosomes are delivered to each spermatid, but one-half of them receives eleven ordinary chromosomes plus the x-chromo-

somes while the other half receives eleven plus the y-chromosome (Figs. 32-34).

In studying the cysts containing spermatids it is very evident that those containing no chromatoid bodies are most numerous, those with one fairly common and those with two or more are much less common. In general, too, the size of these bodies is somewhat less than in the primary spermatocyte although occasionally one large one seems to be retained throughout at its maximum size (Figs. 35-38).

VIII. Summary

1. The spermatogonial number of chromosomes in *Belostoma* (Zaitha) *fluminea* is twenty-four.

2. Only general facts have been determined in regard to synapsis and the post-synaptic stages. During the post-synaptic period a double nature of the chromosome threads is evident.

3. There is a "confused" stage just previous to the prophases of the first division.

4. The chromatoid bodies appear in this "confused" stage for the first time. Proof that they originate from the cytoplasm is not lacking because they are plainly seen outside of the nuclear membrane in this stage.

5. Tetrads appear in the form of rings, Vs, and rods, and all become dumb-bell shaped, by continued condensation, at the time that they enter the spindle.

6. The first maturation division is an equational division. Polar views show thirteen chromosomes, which number is one-half the spermatogonial number plus one.

7. The chromatoid bodies are at their maximum size in this and the following division and generally grow smaller from that point onward.

8. The interkinesis is of short duration. No nuclear vacuole is formed, the chromosomes maintaining their individuality throughout.

9. When the chromosomes arrange themselves in the metaphase of the second division an entirely new arrangement is assumed and an xy-pair of sex-chromosomes can be identified.

10. Twelve chromosomes are delivered to each spermatid in the second division, one-half receive in addition to the eleven ordinary chromosomes an x- and the other half a y-chromosome.

11. The chromatoid bodies behave irregularly all along. Some spermatids have none, others have one and still others in decreasing proportions have two or three.

PAPERS CITED

Fasten, N.

1914. Spermatogenesis of the American Crayfish. Jour. Morph., vol. 25, no. 4.

Morse, M.

1909. The nuclear components of the sex-cells in four species of cockroaches. Arch. f. Zellforsch., Bd. 3.

Wilson, E. B.

1912. Studies on chromosomes. VIII.
Observations on the maturation phenomena in certain Hemiptera and other forms, with considerations of synapsis and reduction. Jour. Exp. Zool. vol. 13.

1913. A chromatoid body simulating an accessory chromosome in Pentatoma. Biol. Bull., vol. 24.

Beloit College.

EXPLANATION OF PLATES

All figures are from *Belostoma* (Zaitha) *fluminea* are drawn with a camera lucida and are magnified about 1350 diameters. Unless otherwise stated the figures are not intended to show *all* the nuclear components.

Plate IX

1-2. Polar views of spermatogonial metaphases, showing twenty-four chromosones.

3. Spermatogonial telophase (probably the last spermatogonial division).

4. Synizesis stage with a plasmosome.

5-6. Post-synaptic nuclei showing the heavy undivided chromosome threads and plasmosomes.

7. A little later stage showing the divided threads. From a smear-preparation. The cell is somewhat distorted.

8. The confused stage, showing three chromatoid bodies outside of the nuclear membrane.

9-9A. About middle prophase of the first spermatocyte division, showing the various forms of the chromosomes during condensation, and the chromatoid bodies.

10. Later prophase of the same division, showing the tetrads and only one chromatoid body.

11. A very clear tetrad.

Plate X

12. A late prophase of the first division showing the chromosomes organized into dumb-bell forms.

13. Very late prophase, showing all the chromosomes and two chromatoid bodies.

14-17. Polar views of metaphases of the first division, showing thirteen chromosomes and either one, two, or no chromatoid bodies.

18-21. Lateral views of the same stage with a varying number of chromatoid bodies.

22-23. Anaphases of the first division illustrating the irregularity in the behavior of the chromatoid bodies during division of the cell.

Plate XI

24. Telophase of the first division. Here the two secondary spermatocytes have each received one chromatoid body. The four small bodies are the controsomes already divided preparatory to the next division.

25. Polar view of the same, showing the crowded condition of the chromosomes.

26-28. Polar views of secondary spermatocytes. All the ordinary chromosomes are here arranged in a circle with the sex-chromosomes in the center and chromatoid bodies without the circle.

29-30. Lateral views of the same, showing the xy-pair of sex-chromosomes in the center.

31. Same stage without the sex-chromosomes.

32. Anaphase of the second division, showing the x- and y- chromosomes going into different spermatoids and slightly in advance of the others.

33-34. Polar views of the same, showing the small y- and the larger x- chromosomes in different spermatids. These cells were somewhat tilted so that in drawing the sex-chromosomes are made to appear displaced from their normal position.

35. Polar view of a spermatid with the chromosomes crowded in telophase, and two chromatoid bodies.

36. Lateral view of the same stage showing one spermatid receiving three chromatoid bodies and the other, none.

37-38. Later views of spermatids just before metamorphosis starts.